CYTOLOGICAL STUDIES OF HEMP AND SANSEVIERIA

Annual Report for 1956

Margaret Y. Menzel

Tallahassee, Florida

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Section of Cotton and Other Fiber Crops

Field Crops Research Branch

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A. CYTOLOGICAL STUDIES OF HEMP

Introduction

In February, 1956, cytological studies of Cannabis sativa were begun with the objects of determining (a) whether bisexual differentiation in this species is accompanied by morphologically-differentiated sex chromosomes, and if so, (b) the sex chromosome constitution of dioecious and monoecious strains being used in the breeding program. It was hoped that these studies could help to explain the rather complex breeding behavior of the monoecious strains.

The author wishes to acknowledge the courtesy of the Department of Biological Sciences, Florida State University, in permitting the use of a phase microscope and greenhouse space, and subsequent to December 1, 1956, for providing laboratory and office space for this work.

General Observations

The following strains of hemp have been studied: Kentucky dioecious (Ky-d); Kentucky monoecious, female habit (Ky-♀m); Kentucky monoecious, male habit (Ky-♂m); German monoecious (G-m); PI 185021; PI 170264; and Wisconsin 372-42. At first, plants were grown in Beltsville and shipped to Tallahassee when almost ready to bloom. Later, material was grown from seed in Tallahassee. Plants of the Kentucky strains planted locally after March 15 were treated to ten hours of light: 14 hours of dark for ten days to induce blooming; untreated control plants from plantings made April 2 did not bloom.

The dioecious strains and Ky-♂m grew well in pot and garden culture and gave good cytological material at least until the end of June. No difficulty arose in classifying the dioecious strains into male and female

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General Observations

The following strains of hemp have been studied: Kentucky dioecious (Ky-d); Kentucky monoecious, female habit (Ky-Sm); Kentucky monoecious, male habit (Ky-om); German monoecious (G-m); PI 189030; PI 170260; and Wisconsin 375-62. At first, plants were grown in Tallahassee and shipped to Tallahassee when almost ready to bloom. Later, material was grown from seed in Tallahassee. Plants of the Kentucky strains planted locally after March 15 were treated to ten hours of light: 14 hours of dark for ten days to induce blooming; untreated control plants from plantings made April 2 did not bloom.

The dioecious strains and Ky-om grew well in pot and garden culture and gave good cytological material at least until the end of June. No difficulty arose in classifying the dioecious strains into male and female

plants. The Ky-♂m plants were more variable in habit than Ky-d males, but none of the plants was classified as having female growth habit. There was much variation in the percentage of pistillate and staminate flowers from plant to plant, and in the ratio on individual plants from beginning to end of blooming.

Ky-♀m also grew fairly well, though not so vigorously as the first five strains, and was rather uniformly female-like in habit. However, the flowers were predominantly female, many plants never producing staminate flowers. Consequently, the number of plants analyzed was low. Of the nine analyzed, one had only staminate flowers, and three had mostly staminate flowers, producing a few pistillate flowers only toward the end of the blooming period.

The phenotypic behavior of the two Ky-m lines in the present study was somewhat at variance with that described by Carl Feaster in his Annual Report for 1955. The differences probably may be attributed to altered expression of certain genotypes under the different and rather unfavorable environmental conditions in Tallahassee.

German monoecious proved very difficult to grow locally. The plants were dwarfed and so variable in habit and flower type that classification was uncertain in many instances. Since they proved to be uniform in the two most important cytological features, their lack of vigor and uniformity probably did not influence the results greatly.

All strains examined had $\underline{n}$ = 10 chromosomes, as reported by previous workers. Meiotic stages in pollen mother cells were studied by the standard aceto-carmine squash technique, following fixation in 3 parts 95% alcohol: 1 part glacial acetic acid and storage in a refrigerator. Pachytene, mid-diakinesis and metaphase I (MI) were favorable for study.

A phase microscope was used to enhance contrast in the prophase stages. A few squashes of roottips were made. However, the mitotic chromosomes were small and rather uniform in appearance. I was unable to distinguish sex chromosomes in these preparations and did not pursue mitotic studies further.

Males of dioecious strains

Although Strasburger and McPhee were unable to distinguish a heteromorphic sex-bevalent in hemp, a number of other workers have reported its presence. In the present work, all males studied at MI from the four dioecious strains had a slightly unequal and asymmetrical one-chiasma bivalent, one of the largest in the complement and frequently lying at the periphery of the plate a little distance from the other bivalents. In a total of 225 cells in which the heteromorphic pair was identified, it always had a single, terminalized chiasma, and usually seemed to be composed of a rod or J-shaped element and a V-shaped element. The asymmetry was more pronounced than the inequality; frequently the rod and the V appeared to be of about the same volume. If unequal, the V was larger. It is not possible, at present, to decide whether the V or the rod is the Y-chromosome.

The heteromorphic pair was regularly detected in Wisconsin 372-42, PI 185021 and PI 170264, but was less reliable in Ky-d for two reasons: (a) the two halves of the bivalent appeared less sharply differentiated in this line, and (b) the lower MI chiasma frequency in Ky-d males, leading to an average of 6 to 7 one-chiasma bivalents per cell, made it more difficult to distinguish the heteromorphic pair from the 1-chiasma autosomal pairs.

At diakinesis in all four strains, however, a heteromorphic pair of chromosomes, usually the largest in the complement, was readily seen under

phase illumination in above 90% of the pollen mother cells. Table 1 compares the ease of detection at MI and diakinesis. One member of the pair had the same "fuzzy" appearance, with somewhat fainter or more diffuse ends, as the other chromosomes. The other member had one such end, in which at least one chiasma was invariably present in the 565 cells (Table 1) in which it was identified. The remainder of this chromosome was sharply outlined and more deeply stained; the "heterochromatic" end was usually abruptly defined and did not taper out into faint threads as did the other ends. Since only dioecious males possessed the partially heterochromatic chromsome, it is assumed to be the Y-chromosome.

One other chromosome pair, the smallest, could regularly be identified at diakinesis by its attachment to the nucleolus. It characteristically appeared as a ring (2-chiasma) bivalent with two small heterochromatic "ears", distal to one of the chiasmata, attached to the nucleolus. The ears had the same optical and staining properties as the heterochromatic portion of the Y.

All of the 43 males studied had the same karyotype and may be assumed to be XY (Table 2).

Females of dioecious strains

On April 12, 1956, 29 potted plants of Ky-d, already in bloom, were received from Beltsville. The pots were sunk in the ground in the experimental garden, and the plants were classified as 15 ♂: 14 ♀. On May 17, it was observed that five of the females were producing a few staminate flowers. All of the females were heavily loaded with seed at this time and had almost ceased growth.

The staminate buds were collected and pollen mother cells at diakinesis were obtained from plant No. 75. All the bivalents were of similar chromaticity; the heterochromatic chromosome was not present. The nucleolar pair was similar to that in the males. It may be assumed,

phase illumination in above 90% of the pollen mother cells. Table 1 compares the case of detection at MI and diakinesis. One member of the pair had the same "fuzzy" appearance, with somewhat farther or more diffuse ends, as the other chromosome. The other member had one such end, in which at least one chiasma was invariably present in the 595 cells (Table 1) in which it was identified. The remainder of this chromosome was sharply outlined and more deeply stained; the "heterochromatic" end was usually sharply defined and did not taper out into fetal threads as did the other ends. Since only dioecious males possessed the partially heterochromatic chromosome, it is assumed to be the Y-chromosome.

One other chromosome pair, the smallest, could regularly be identified at diakinesis by its attachment to the nucleolus. It characteristically appeared as a ring (2-chiasma) bivalent with two small heterochromatic "arms", distal to one of the chiasmata, attached to the nucleolus. The arms had the same optical and staining properties as the heterochromatic portion of the Y.

All of the 43 males studied had the same karyotype and may be assumed to be XY (Table 2).

Females of dioecious strains

On April 12, 1956, 29 potted plants of Hy-4, already in bloom, were received from Beltsville. The pots were sunk in the ground in the experimental garden, and the plants were classified as 15 ♂: 14 ♀. On May 17, it was observed that five of the females were producing a few staminate flowers. All of the females were heavily loaded with seed at this time and had almost ceased growth.

The staminate buds were collected and pollen mother cells at diakinesis were obtained from plant No. 25. All the bivalents were of similar chromaticity; the heterochromatic chromosome was not present. The nucleolar pair was similar to that in the males. It may be assumed,

therefore, that the female was of XX constitution (Table 2).

It was observed on June 12 that the 15 males and the 9 females which had not produced staminate flowers were dead or dying. The five females which had produced staminate flowers had, however, begun to put out lateral, vegetative branches. In hopes that they might produce additional staminate buds, they were maintained throughout the summer and grew into very large, bushy plants, two reaching a height of over eight feet. They did not produce buds again until the end of October. When the plants were destroyed early in November, all branches were carefully examined for staminate buds, but only hundreds of pistillate buds were found.

Kentucky female-habit monoecious and German monoecious

Meiotic stages were found in 9 Ky-♀m and 15 G-m plants (including 3 male habit, male-flowered plants of the latter). All 24 plants, regardless of habit or type of flowers, had only equal, symmetrical, "euchromatic" bivalents, similar to those in Ky-d female No. 75 (Table 2). Since Ky-♀m and G-m karyotypes were indistinguishable from each other and from that of No. 75, and differed from that of the dioecious males, and since Ky-♀m arose by selection from the progeny of dioecious females which had produced a few staminate flowers, it is concluded that the Ky-♀m and the G-m plants were all XX.

With the exception of one plant (No. 31) of Ky-♀m, the female monoecious plants all had the characteristic nucleolar pair described above, a euchromatic ring with two small, distal, heterochromatic ears. The nucleolar pair of No. 31 had the peculiarities at both diakinesis and MI to be described in the next section.

Kentucky male-habit monoecious

Plants were grown from sibbed and selfed seeds from four male-habit monoecious lines which had appeared in a field of Ky-♀m. Since no

differences in karyotype were found between the four lines, or between sibbed and selfed progenies, they will all be considered together.

In all 21 Ky-♂m plants analyzed (Table 2), the nine non-nucleolar bivalents were equal and symmetrical at MI and euchromatic at diakinesis. They must therefore be presumed to be of the sex genotype XX.

The nucleolar bivalent at diakinesis in all 21 plants differed from that in all other plants studied (except Ky-♀m No. 31) in that the ears were replaced by a very large heterochromatic structure attached to the nucleolus. The "knob" plus the bivalent often looked like a chain trivalent composed of three approximately equal members. The knob persisted until metaphase I as a single or a pair of satellite-like structures attached by a thread or a constriction to one of the smaller bivalents. Rarely, it was detached and appeared as a small univalent or free fragment. It was sometimes seen as either a fragment or a satellite at AI, but in most AI cells it was not visible, only ten bodies being present.

The possibility that the knob represented an extra chromosome or pair of chromosomes was considered, but its history from pachytene (where it appeared as a large, deeply-stained loop or pair of loops on the nucleolus, continuous with the rest of the nucleolar pair) to MII (when it was no longer visible) indicated that it is not a separate centric element but rather a large heterochromatic region associated with the nucleolus organizer. Some details of its behavior (e.g., its appearance as the end member of a chain of three bodies at diakinesis versus its frequent appearance at MI as two separate bodies attached to opposite ends of a small rod bivalent) are not understood at present.

Origin of male-habit plants from female-monoecious lines

It seems rather certain that the male habit plants which have arisen in both G-m and Ky-♀m cannot be due to the re-introduction of a Y chromosome by accidental outcrossing to dioecious males. They must arise from segregation within the respective lines themselves.

In G-m, segregation of male plants was not accompanied by detected cytological changes, and is presumably strictly genic. Breeding behavior of G-m males, so far as I know, has not been tested.

The Ky-♂m plants, on the other hand, apparently breed more or less true, both for male habit and for the large nucleolar knob. Since the knob is heterochromatic at diakinesis like part of the Y chromsome, it is tempting to suppose that it is a sort of "neo-Y" (Y^n) chromosome, perhaps having arisen (a) by translocation of a portion of the Y controlling ♂ habit to the nucleolar chromosome, or (b) by the acquisition by the nucleolar chromsome of an extra block of heterochromatin (non-homologous with Y) which per se shifts growth habit toward maleness, at least when homozygous. In either case, the Ky-♂m plants would be XX Y^nY^n, which might help to explain constancy for male habit as opposed to the variability of flowering habit.

Crosses of Ky-♂m X Ky-d♂ are being grown to try to recover hybrids having both "knob" and "Y". A study of diakinesis in such hybrids should answer the question of whether the two are partially homologous.

The finding of a female habit Ky-♀m plant (No. 31) with the nucleolar knob and only staminate flowers challenges the "Y^n" hypothesis, although it does confirm that the knob is present in the Ky-♀m line and that the true-breeding knobs of the Ky-♂m plants could have arisen by segregation. Perhaps plant No. 31 was heterozygous for knob (XX Y^n-), which might explain its phenotypic difference from Ky-♂m. Unfortunately, the plant died

Origin of male-habit plants from female-monoecious lines

It seems rather certain that the male habit plants which have arisen in both O-m and Ky-Pa cannot be due to the re-introduction of a Y chromosome by accidental outcrossing to dioecious males. They must arise from segregation within the respective lines themselves.

In O-m, segregation of male plants was not accompanied by detected cytological changes, and is presumably strictly genic. Breeding behavior of O-m males, so far as I know, has not been tested.

The Ky-Pa plants, on the other hand, apparently breed more or less true, both for male habit and for the large nucleolar knob. Since the knob is heterochromatic at diakinesis like part of the Y chromosome, it is tempting to suppose that it is a sort of "neo-Y" (Y_n) chromosome, perhaps having arisen (a) by translocation of a portion of the Y controlling c habit to the nucleolar chromosome, or (b) by the acquisition by the nucleolar chromosome of an extra block of heterochromatin (non-homologous with Y) which per se shifts growth habit toward maleness, at least when homozygous. In either case, the Ky-Pa plants would be XX $Y_n Y_n$, which might help to explain constancy for male habit as opposed to the variability of flowering habit.

Crosses of Ky-Pa X Ky-Pa are being grown to try to recover hybrids having both "knob" and "Y". A study of diakinesis in such hybrids should answer the question of whether the two are partially homologous.

The finding of a female habit Ky-Pa plant (No. 31) with the nucleolar knob and only staminate flowers challenges the "Y_n" hypothesis, although it does confirm that the knob is present in the Ky-Pa line and that the true-breeding knobs of the Ky-Pa plants could have arisen by segregation. Perhaps plant No. 31 was heterozygous for knob (XX Y_n-), which might explain its phenotypic difference from Ky-Pa. Unfortunately, the plant died

before its significance was recognized. Attempts should be made to recover other "knobbed" plants from Ky-♀m so that progeny tests and more detailed cytological studies may be made.

The possibility cannot be entirely excluded at present that the simultaneous "homozygosity" for non-XY male habit and for knob in all the Ky-♂m lines studied is purely coincidental. Appropriate crosses to clarify this point are planned for 1957.

Location of the differential segments and of chiasmata in the XY chromosome pair.

Pachytene:

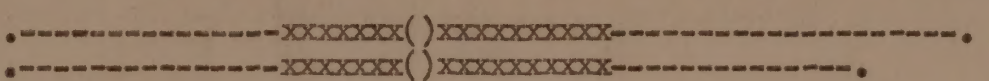
With a view to determining the extent and position of the homologous and differential segments of the X and Y chromosomes in dioecious males, a preliminary examination of pachytene was made. Mid-pachytene chromosomes of hemp are composed of relatively deeply-staining or "condensed" segments proximal to the centromere (which appears as a gap or constriction), passing rather abruptly into long, faint "tails", similar to those figured for tomatoes by Brown and Barton. In hemp, most (all?) of the tails terminate in small, deeply-stained dots or "telomeres", which facilitates tracing out individual pairs to their ends. The greatest difficulty lay in spreading the chromosomes sufficiently to follow all the long, tangled pairs without disrupting the nucleus entirely. Only one nucleus has been seen so far in which all ten bivalents could be followed. Many nuclei were found, however, in which one to several pairs could be traced from end to end.

Eight of the pachytene bivalents have distal tails on both arms. The nucleolar pair has a tail only on the arm opposite the nucleolus organizer. One other bivalent regularly appeared to have a tail on only one arm. The length of the condensed regions appeared to increase relative to that of

the tails from early to late pachytene.

In one of the two-tailed pachytene bivalents, one of the arms (which will be designated the "right" or "R" arm) had one tail slightly longer than the other. Since this bivalent was the only one which showed any differentiation between the members, it is taken to be the XY pair. Present evidence does not indicate which is X and which Y. The unequal pair was not found in three Ky-♀m, 1 G-m and 4 Ky-♂m plants which were studied at pachytene.

In all 18 nuclei in which the unequal pair was identified, all other bivalents appeared to be completely synapsed from end to end. Synapsis in the four regions of the unequal pair was as follows:



	L_d	L_p	R_p	R_d
complete*	18	13	18	5
partial		5		5
absent				8

* "Complete", with reference to the distal region of the right arm (R_d), means that synapsis has proceeded out to the telomere of the shorter arm, leaving a short, single strand of the longer arm projecting at the end.

The R_d region evidently contains a differential segment. Since complete synapsis of the shorter tail occurred in 5 cells, it was at first thought that only the extreme distal end of R_d was differential. However, McClintock has convincingly demonstrated (in corn) that chromosome segments which lack homologues have a strong tendency to synapse non-homologously. Non-homologous pairing can be just as complete and intimate

as homologous pairing, but is less constant in occurrence. Since synapsis of R_d was incomplete in 5 nuclei and absent in 8, it seems likely that the entire R_d segments are differentiated and that a variable amount of non-homologous pairing occurs between them. In three of the five completely synapsed bivalents, it could clearly be seen that the telomere of the shorter chromosome arm was not matched by a similar chromomere in the opposite chromsome.

The occurrence of a short unpaired region in L_p in five cells may mean that these segments are also partially differentiated.

McClintock found that non-homologous pairing seldom persists until diakinesis. However, in rare instances, "crossovers" leading to new chromosome types probably take place following such pairing. This should be borne in mind in the discussion of chiasma number and position in the XY pair at diakinesis (see below).

Diakinesis:

It has been suggested that the XX pair in monoecious lines may not be identical with the X's of the dioecious females, but rather the product of rare crossing-over between the differential segments of X and Y (leading, for instance, to insertion into the X of a Y segment affecting flower type but not growth habit). This hypothesis (which will be referred to as the X^y hypothesis) is not invalidated by the data which led to assumption of an XX constitution for the monoecious lines, since such a transposed Y segment might be too small to be detected cytologically, or might lose its differential chromaticity when inserted into the "euchromatic" X, while still retaining its genetic potency.

To see whether either support or disproof of the X^y hypothesis could

be obtained, the configuration of the XY pair at diakinesis was tabulated in 540 cells. The six configurations observed are shown diagrammatically in Table 3, together with their frequencies.

The positions of the centromeres could not be determined at diakinesis, and since the XY pair could not be followed through its diplotene evolution, the correspondence of parts between the pachytene and diakinetic XY bivalent was not established with certainty. Two inferences seem justified by the available evidence, however; (a) The heterochromatic region of the Y at diakinesis does not coincide with the L_p and R_p regions of pachytene, though it may overlap them. (b) The euchromatic portion of the Y at diakinesis and the corresponding portion of the X represent the left arm (probably L_d) of the pachytene bivalent.

In 6% of the cells (Table 4) the configuration of the XY could not be determined, usually because it was partially obscured by another bivalent or by the nucleolus. There was no reason to suppose that any of the undetermined bivalents had configurations other than the six types listed.

All of the 94% of the XY bivalents in which the position of chiasmata could be determined had a chiasma in the L arm. In 78%, this was the only chiasma present. An additional 3% had a second chiasma in this region and no other. In these cells, the unpaired arms of the XY were usually widely separated, the bivalents appearing as a straight rod or a broad "U", with the chiasma often stretched as if the two chromosomes were actually repelling each other more strongly than the members of the other bivalents. The contrast between configurations of types 1 and 2a and types 2b, 2c and 3 was therefore more marked than is indicated in the diagrams. In the best preparations, a definite suggestion of a cross-

shaped configuration could be seen at the points of contact in the L arm, so that it seems certain that they were true chiasmata.

The configurations of types 2b, 2c and 3 (a total of 12%) were more difficult to interpret. In contrast to types 1 and 2a, the differential segments showed either a terminal (2b) or an interstitial (2c, 3) contact in the right arm, and opened out on either side of the contact point, suggesting chiasmata. On the other hand, no visible evidence of a change of partners was noted; rather, the contact usually appeared to be merely in the form of a half-twist between the chromosomes. Moreover, assuming that the differential chromaticity of the Y is an autonomous property of each chromatid, true chiasmata in the differential region should lead to adjacent loops, one of which is heterochromatic on only one side and the other on both sides. No such configurations were seen. It seems more likely, therefore, that the points of contact between the X and Y right arms are not true chiasmata, but rather half-twists of relational coiling, probably left over from non-homologous synapsis at pachytene.

No evidence was found, therefore, in support of the X^y hypothesis, although it is not ruled out altogether by the data. It may be possible to test this hypothesis more rigorously by comparing diakinetic configurations in XY plants from crosses of monoecious X ♂ dioecious with those in dioecious males. An increase in contacts in the right arms in the hybrids, or the finding of true chromatid exchange at such points, would constitute evidence for increased homology and, therefore, for the existence of an X^y chromosome in the monoecious parents.

Chiasma frequencies in various strains

Examination of meiotic stages in various hemp lines gave the

impression that there were strain differences in chiasma frequency. Table 4 summarizes the actual chiasma counts recorded from slides deemed clear enough for the purpose. While these data are insufficient to permit definite conclusions, they tend to confirm certain impressions gleaned from a much larger sample studied primarily for other reasons:

1. Ky-d males average 2-3 more 1-chiasma bivalents at MI than do the other strains studied. As pointed out above, this increases the difficulty of identifying the XY pair.

2. There seems to be a tendency for chiasmata to terminalize between diakinesis and MI in Ky-d males, whereas in the other lines the chiasma counts for diakinesis and MI are similar.

3. Three plants of G-m (Tables 4 and 5) were partially "asynaptic", having 0-10 univalents at MI. In 159 cells of Belts. ♂ No. 2, 67 cells (42%) had 2-10 univalents, with an average of 1.4 I/cell, yet the overall MI chiasma frequency was 1.31 per bivalent, only a little lower than the average for Ky-d males, which rarely have univalents at MI. Moreover, in 11 cells at diakinesis, no univalents were seen and the chiasma frequency was 1.70 per bivalent. These data suggest (a) that the univalents are due to premature terminalization of chiasmata before MI ("desynapsis") rather than to failure of synapsis or chiasma formation, and (b) that the premature terminalization is not at random, but affects some chromosome pairs more often than others.

4. Although two of the desynaptic plants were strict males, plant No. 37 was a female monoecious plant. Furthermore, Belts. ♂ No. 1 had the highest chiasma frequency of any plant studied at both diakinesis and metaphase I (Table 3). Eighteen of 79 MI cells (23%) had 10 ring bivalents, incidentally confirming its homozygosity for the sex chromosome pair. Apparently desynapsis is not associated with sex determination.

5. Cells with 10 ring bivalents were also observed occasionally in other G-m, Ky-♀m and Ky-♂m plants, but never in dioecious males, further supporting the conclusion that the former are XX and the latter XY.

Table 1. Comparison of number of cells in which the heteromorphic bivalent was identified at metaphase I and at diakinesis.

Strain (males)	No. Plants	Metaphase I				Diakinesis			
		Yes	No	Total	% Yes	Yes	No	Total	% Yes
PI 185021	6	22	-	22	100	89	1	90	99
PI 170264	2	5	-	5	100	19	1	20	95
Wisc. 372-42	12	12	1	13	92	133	5	138	96
Ky-d	20	186	59	245	76	324	25	349	93
Total	40	225	60	285	79	565	32	597	95
Ky-d selected *	5	125	54	179	69	157	11	168	93

* A special sample in which particular care was taken to record all cells in which ten bivalents could be counted, whether or not the heteromorphic bivalent was seen.

Table 1. Comparison of number of cells in which the heteromorphic bivalent was identified at metaphase I and at diakinesis.

Strain (males)	No. Plants	Metaphase I				Diakinesis			
		Yes	No	Total	% Yes	Yes	No	Total	% Yes
PI 185021	6	22	-	22	100	89	1	90	99
PI 170268	2	5	-	5	100	19	1	20	95
Wisc. 372-42	12	12	1	13	92	123	5	128	96
Ky-4	20	186	59	245	76	324	25	349	93
Total	40	225	60	285	79	565	32	597	95
Ky-4 selected *	5	125	54	179	69	157	11	168	93

* A special sample in which particular care was taken to record all cells in which ten bivalents could be counted, whether or not the heteromorphic bivalent was seen.

Table 2. Summary of lines and plants analyzed for the presence or absence of the heteromorphic bivalent and of the nucleolar knob.

Strain	No. Plants	Habit	Flowers	Het. II	Knob	Sex Genotype
Dioecious						
Wisc. 372-42	12	♂	♂	yes	no	XY
PI 185021	6	♂	♂	yes	no	XY
PI 170264	2	♂	♂	yes	no	XY
Ky-d	23	♂	♂	yes	no	XY
Total -	43					
Ky-d No. 75	1	♀	♀(♂)*	no	no	XX
Monoecious						
G-m	3	♂	♂	no	no	XX
	1	♂	♀♂	no	no	XX
	5	♀	♂	no	no	XX
	6	♀	♂(♀)**	no	no	XX
Total -	15					
Ky-♀m	5	♀	♀♂	no	no	XX
	3	♀	♂♀	no	no	XX
(No. 31)	1	♀	♂	no	yes	XX
Total -	9					
Ky-♂m	4	♂	♂	no	yes	XX
	17	♂	♀♂	no	yes	XX
Total -	21					

* Flowers were predominantly ♀ until late in blooming period, when a few ♂ flowers were produced.

** Flowers were predominantly ♂ until late in blooming period, when a few ♀ flowers were produced.

Table 2. Summary of lines and plants analyzed for the presence or absence of the heterozygote bivalent and of the nucleolar knob.

Strain	No. Plants	Habit	Flowers	Het. II	Knob	Sex Genotype
Dioecious						
Wisc. 372-32	12	d	d	yes	no	XX
PI 180021	6	d	d	yes	no	XX
PI 179056	2	d	d	yes	no	XX
Sp-d	23	d	d	yes	no	XY
Total -	43					
Sp-d No. 75	1	9	9(1)ss	no	no	XX
Monoecious						
G-m	3	d	d	no	no	XX
	1	d	99	no	no	XX
	5	9	d	no	no	XX
	6	9	d(9)ss	no	no	XX
Total -	15					
Sp-Gm	5	9	99	no	no	XX
	3	9	d9	no	no	XX
(No. 31)	1	9	d	no	yes	XX
Total -	9					
Sp-Gm	4	d	d	no	yes	XX
	17	d	99	no	yes	XX
Total -	21					

* Flowers were predominantly o until late in blooming period, when a few o flowers were produced.

** Flowers were predominantly o until late in blooming period, when a few 9 flowers were produced.

Table 3. Configurations of the XY bivalent observed at diakinesis and their frequencies.

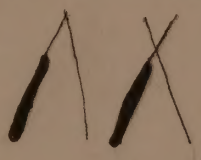
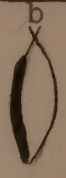
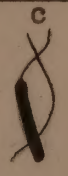
Strain (males)	No. Plants	1-chiasma 	2-chiasma? a	b 	c 	3-chiasma?	Not det.	No. cells
Ky-d	19	290	8	4	34	5	30	371
PI 170264	1	9	-	-	--	-	1	10
PI 185021	3	43	3	-	-	-	1	47
Wisc. 372-42	11	81	5	3	17	1	5	112
Total	34	423	16	7	51	6	37	540
Frequency		.78	.03	.01	.09	.01	.06	

Table 4. Chiasma frequencies at diakinesis and metaphase in various hemp strains. For the present purpose, the XY pair was tabulated as having only one chiasma in each cell.

Strain	Plant No.	Diakinesis			Metaphase I			Difference (D-M)
		No. II	No. Xm	Xm/II	No. II	No. Xm	Xm/II	
Ky-d ♂♂	1	140	251	1.79	150	238	1.59	.20
	2	40	60	1.50	170	229	1.35	.15
	3	270	495	1.83	60	90	1.50	.33
	11	1000	1537	1.54	1000	1274	1.27	.27
	12	250	371	1.48	100	150	1.50	-.02
	18	290	438	1.51	280	432	1.54	-.03
Total	6	1990	3152	1.58	1760	2413	1.37	.21
Ky-♀m	31	80	143	1.79	120	201	1.67	.12
Ky-♂m	46	290	537	1.51	170	261	1.53	-.02
	45	-	-	-	90	159	1.77	-
	49	-	-	-	90	144	1.60	-
Total	3	290	537	1.51	350	564	1.61	-.10
G-m normal	98	-	-	-	160	268	1.67	-
	(99,123, 124,125)	-	-	-	250	325	1.30	-
Belts. ♀ mon		100	168	1.68	810	1401	1.73	-.05
Belts. ♂ mon		390	539	1.38	360	484	1.34	.04
Belts.♂ No.1		230	438	1.90	790	1435	1.82	.08
Total	8	720	1145	1.59	2370	3913	1.65	-.06
G-m desynaptic Belts.♂ No.2		110	187	1.70	1590	2084	1.31	.29
	37	-	-	-	40	48	1.20	-
Total	2	110	187	1.70	1630	2132	1.31	.29

Table 1. Distance frequencies at diakinesis and metaphase in various heterozygotes. For the present purpose, the XX pair was tabulated as bearing only one chiasma in each cell.

Stock	Plant No.	Diakinesis No. II	Diakinesis No. ring	Diakinesis Chi/II	Metaphase I No. II	Metaphase I No. ring	Metaphase I Chi/II	Difference (D-M)
Dy-4 dd	1	340	251	1.79	350	238	1.59	.20
	2	40	30	1.50	370	279	1.35	.15
	3	270	195	1.83	80	90	1.30	.33
	11	1000	1537	1.54	1000	1274	1.27	.27
	12	290	371	1.48	300	150	1.50	-.02
	18	290	198	1.51	280	132	1.34	-.03
	Total	6	1990	1432	1.39	1760	2429	1.37	.21
Dy-Sa		31	80	145	1.79	120	202	1.67	.12
Dy-ca		16	290	537	1.52	170	264	1.53	-.02
		15	-	-	-	90	159	1.77	-
		19	-	-	-	90	144	1.60	-
	Total	3	290	537	1.52	350	564	1.61	-.10
O-a normal		38	-	-	-	160	268	1.67	-
		124	-	-	-	250	325	1.30	-
	Delta. 9 mon	120	168	1.50	810	1402	1.73	-.05
	Delta. 6 mon	290	359	1.38	360	404	1.34	.04
	Delta. No.2	230	168	1.30	720	1152	1.60	.00
	Total	8	720	1115	1.39	1370	2312	1.68	-.06
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		37	-	-	40	48	1.20	-
	Total	2	120	187	1.70	1630	2132	1.31	.39

Table 5. Summary of metaphase I data on three slightly desynaptic plants of G-m.

Plant No.	Habit	flowers	No. cells	No. cells with I's	No. I's	I/cell	% cells with I's
37	♀	♀♂	4	4	20	5.0	100
93	♂	♂	90	38	112	1.1	42
Belts. ♂ No.2	♂	♂	159	67	228	1.4	42

Table 5. Summary of metaphase I data on three multiply deficient plants of C-n.

Plant No.	Habit	Flowers	No. cells	No. cells with I's	No. I's	I/cell	% cells with I's
27	♀	♀	4	4	20	5.0	100
93	♂	♂	90	38	112	1.1	42
Baite. ♂ No.2	♂	♂	159	67	228	1.4	42

B. CYTOLOGICAL STUDIES OF SANSEVIERIA

Introduction

In connection with the breeding program at the Everglades Experiment Station, a cytological survey of 21 species and several species hybrids of Sansevieria was begun in September, 1956. The immediate objectives were to determine: (a) the basic chromosome number or numbers (x) of the genus; (b) the extent and identity of polyploid species and races, if any; (c) the relationships between species in so far as could be inferred from karyotypes and from chromosome behavior in the available species hybrids.

Materials and Methods

Plants for studies of mitotic chromosome number and morphology in roottips were sent to Tallahassee from Belle Glade. The best roottips were obtained from rhizomes and leaf cuttings rooted in moist, finely-divided expanded Perlite Aggregate ("Permalite"). Rapidly growing roots were excised, placed in petri dishes containing filter paper wet with a saturated solution of para-dichloro-benzene (PDB) for 1 1/2 to 3 hours, fixed in 3 parts 95% alsohol: 1 part glacial acetic, macerated for 1 minute in 10% HCl, and squashed in the usual way in acetocarmine. A critique of this procedure will be given below.

Buds were collected at the Everglades Experiment Station by Dr. James B. Pate and by the author and shipped to Tallahassee for study. The technique was the same as above, except that PDB pretreatment and maceration were omitted.

The mitotic studies were initiated first, since buds did not become available until December. It was expected that chromosome numbers and

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Plants for studies of mitotic chromosome number and morphology in rootTips were sent to Tallahassee from Belle Glade. The best rootTips were obtained from rhizomes and leaf cuttings rooted in moist, finely-divided expanded Perlite Aggregate ("Permalite"). Rapidly growing roots were excised, placed in petri dishes containing filter paper wet with a saturated solution of para-dichloro-benzene (PDB) for 1 1/2 to 3 hours, fixed in 3 parts 95% alcohol: 1 part glacial acetic, macerated for 1 minute in 10% HCl, and squashed in the usual way in acetocarmine. A critique of this procedure will be given below.

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The mitotic studies were initiated first, since buds did not become available until December. It was expected that chromosome numbers and

gross differences in karyotype between species could be determined in this way. Since mitotic counts gave highly equivocal results, the meiotic studies will be discussed first.

Meiotic Chromosomes

Pachytene, diplotene, diakinesis, metaphase I and a few anaphase I stages were surveyed.

Pachytene:

Pachytene chromosomes in Sansevieria are adequately stained and in diploid species can be seen to be composed of two parallel strands with deeply staining proximal segments fading distally into faint "tails". Neither the extent of the proximal regions nor the positions of the centromeres were so clearly defined as in hemp. Differentiated telomeres were not observed in Sansevieria. The high chromosome number makes a complete pachytene analysis impracticable, but two interesting features were noted.

(1). In several diploid species, and in a diploid trifasciata-deserti F_1, all the chromosomes were synapsed from end to end. In an approximately triploid trifasciata-deserti hybrid, on the other hand, three-stranded (trivalent) figures and some unpaired chromosomes were noted. The observations agreed with metaphase I data for the respective forms.

(2). The nucleolus at pachytene in trifasciata-deserti F_1 H51-10 was a two-parted structure composed of a small and a large sphere tangent to each other. The two spheres joined at a heterochromatic knob, probably the nucleolus-organizer, which could be seen to be interstitially located in the shorter arm of a submetacentric pair of chromosomes. A few observations in pure species suggested that this rather unusual appearance of the

pachytene nucleolus is characterstic of the genus rather than of H51-10 in particular.

Diplotene and diakinesis:

At diplotene, the proximal and distal segments are more sharply differentiated than at pachytene, but the centromere was not visible. Individual bivalents were seen to be opened out into typical loops between chiasmata. The nucleolus at this stage was a single large sphere. By mid-diakinesis it had begun to fragment and disappear. At mid- and late diakinesis, the bivalents were easily counted, but were so compact and densely stained that their structure could not be studied.

Metaphase I:

The first metaphase division was most favorable for the purposes at hand. Table 1 summarizes data on chromosome numbers and pairing in the 11 species from which suitable material has been collected. Table 2 gives similar data from five types of species hybrids.

Although only about half the available species representing perhaps 15% of the described species have been examined at meiosis, and data from some of these are as yet fragmentary, the work has progressed far enough to permit formulation of a tentative hypothesis concerning the cytotaxonomic structure of the genus.

(1). The lowest chromosome number found (in 8 of the 11 species studied) was $n = 20$. That this represents the true basic number in Sansevieria is supported by the regular presence of 20 bivalents in MI; no multivalents were seen in any of the diploid species.

(2). Three of the 11 species are polyploid. S. canaliculata is tetraploid ($n = 40$); 1-5 IV's were found at MI. The rest of the chromosomes

were paired as bivalents. Only occasional cells had III's and I's, and no association of more than four chromosomes was seen.

S. subspicata is hexaploid (n = 60), and IV's and VI's are found at MI. Very few cells had unpaired chromosomes.

S. liberica is approximately tetraploid. Exact counts were difficult both because material from this species was poorly fixed, and because pairing was less regular than in the other two polyploids. Trivalents and univalents were more common than in the other two, but IV's were the highest associations seen.

(3). Diploid hybrids of trifasciata x deserti, trifasciata x parva, and parva x deserti regularly had 20 II at MI, and no suggestion was seen that the chiasma frequency was lower than in the parental species. It appears that the genomes of all three species are similar.

(4). The similar meiotic karyotypes of all the diploid species suggests that the other five diploid species also have genomes similar to those of trifasciata, deserti and parva.

(5). The cytological details of the multivalents in S. canaliculata and S. subspicata indicate that they are due to the presence of four and six sets of homologous chromosomes, respectively (not to heterozygosity for chromosome rearrangements). These forms must be either autoploids, or amphiploids derived from closely related ancestors. A priori, the latter is considered to be more likely.

(6). Liberica seems to have less regular pairing than the other two polyploids, and its behavior suggests either that it has 3 similar and 1 dissimilar sets of chromosomes, or that factors for asynapsis are present. In the absence of other evidence for genome differentiation in the genus,

the latter interpretation appears more likely at present.

(7). As expected from the chromosome numbers of the parents, the two *trifasciata* x *liberica* F_1's had approximately the triploid number. The two hybrids differ in phenotype. H51-1, the lighter-green, more *trifasciata*-like of the two, was hypotriploid, while the darker, more *liberica*-like H51-2 probably had more than 60 chromosome (hypertriploid). The phenotypic differences probably reflect a higher proportion of *liberica* chromsomes in the latter plant. Variation in chromosome number in *liberica* gametes is to be expected from the meiotic irregularities in the species.

(8). A group of nine *trifasciata* x *deserti* hybrids from two crosses made by Dr. Pate in 1954 differed strikingly in appearance from other F_1's of this cross (of which diploid H51-10 was representative). The H-54 series of hybrids were much larger, with broader leaves, and more variable than other groups of F_1's. Aside from their *gigas* characters, they appeared more or less intermediate between the ordinary F_1's and *trifasciata*, leading to the prediction that they were triploids with two *trifasciata* and one *deserti* genome ("TTD"). Analysis of four of the aberrant hybrids showed them to have approximately the triploid number; probably there was some variation in number from plant to plant. Variation in number would be expected if the hybrids arose, as postulated, from autopolyploid sectors of *trifasciata*, and may explain the greater variation among the 3x F_1's as compared with the more uniform diploid hybrids ("TD").

The possibility cannot be excluded on cytological grounds that the hybrids received the double set of chromosomes from *deserti* and are TDD in constitution, but their phenotypes make this the less likely of the two possibilities.

(9). The extra vigor of the TTD as compared with the TD F_1's (which themselves show some heterosis) indicates that polyploid as well as hybrid vigor can operate in Sansevieria, and suggests a wide range of breeding possibilities if tetraploid forms of trifasciata and perhaps of other diploid species and hybrids can be established.

(10). Both the trifasciata x liberica and the 3x trifasciata x deserti hybrids showed a high frequency of trivalents at MI. In the latter, an average of about 13 III per pollen mother cell (out of a possible 20) was found. Moreover, the III's were frequently complex, with 3, 4 or more chiasmata, rather than simple V's. The high III frequency and high chiasma frequency (a) greatly complicate determination of the exact chromosome number, and (b) indicate a high degree of homology between the parental genomes.

(11). Dr. Pate has classified the species of Sansevieria into three groups according to leaf type: Group I, round-leaved; Group II, coriaceous; Group III, flat, petiolate leaves. These morphological catagories do not appear to coincide with any significant differentiation in karyotype, or with marked barriers to gene exchange. The data do suggest that natural polyploidy may be more common in Groups I and II than in Group III, but a much larger sample of species from each group is needed to establish this point.

It should, of course, be borne in mind that the conclusions presented above are tentative, and may be modified as additional species and hybrids become available for study.

Mitotic Chromosome Morphology and Numbers

It early became apparent that interpretation of roottip chromosome numbers must await analysis of meiosis. A complete resume of data on mitotic numbers will not be given in this report. Three aspects of these studies will be noted briefly.

(1). Roottip divisions were studied from all 21 species. Too few divisions were seen from abyssinica and angustiflora to warrant discussion. In the other 19 species, the only significant difference in karyotype was in chromosome numbers. All species had the same range of chromosome sizes and types. This finding agrees with the conclusion from meiotic data concerning the lack of cytological differentiation of genomes in Sansevieria.

(2). The basic number in roottips of all species except singularis (see below) appeared to be $x = 21$. When it was found that x clearly equals 20 in the pollen mother cells, roottip preparations were re-examined in search of an explanation of the discrepancy. It had been noted earlier that each of the two smallest "chromosomes" in mitotic plates often lay at the end of another, medium-sized chromosome. Since no connection could be seen between the two, they were interpreted as separate elements. It appears likely that the "smallest chromosomes" are actually relatively large satellites set off by marked constrictions from the rest of the chromosome. This interpretation is supported by two observations: (a) The nucleolus organizer appears at pachytene to be interstitial, so that the nucleolar pair might be expected to show a large satellite at mitosis. (b) The "small chromosomes" are about 1/2 the size of the next largest pair at mitosis, but no such very small bivalent can be identified at MI, suggesting that it is this "smallest mitotic pair" which is missing at meiosis and, hence, is not actually a pair of separate elements.

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This situation may explain disagreements in the literature as to whether x = 20 or 21 in the genus.

(3). The most striking feature of mitosis in Sansevieria was the high variability in chromosome number in some species.

(a) In most species in which 25 or more cells were counted, either polyploid or reduced cells, or both, were found.

(b) Except in singularis, in which the numbers were so variable that both the x and the n numbers remain undetermined, the numbers fell mostly into a euploid series.

(c) The counts did not, however, fit the simple 2^n series to be expected if some cells had undergone chromosome doubling (e.g., as a prelude to differentiation). Instead, the following numbers were found: x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 10x, 12x. Occasional cells, which clearly appeared to be intact, had less than x, e.g., 7, 9, 11, 14, 15 chromosomes. Cells with 28 to 30 chromosomes were seen in some species. The numbers 2x, 4x, 6x were most frequently encountered.

(d) In some species in which the chromosomes have been counted in both roottips and pollen mother cells, most roottip cells had the 2n number. For instance:

		No. cells with					
species	PMC's	x	2x	3x	4x	6x	8x
trifasciata	n(x) = 20	-	30	2	3	-	-
metallica	n(x) = 20	2	50	1	7	-	-
canaliculata	n(2x) = 40	-	-	-	15	2	-
subspicata	n(3x) = 60	-	-	1	2	10	1

(e) In other species, numbers other than 2n were so common as to give an erroneous estimate of ploidy of the species before the true n number had

species	Refs.	x	$2x$	$3x$	$4x$	$6x$	$8x$
trifasciata	$n(x) = 20$	–	20	2	3	–	–
reflexa	$n(x) = 20$	2	20	1	7	–	–
caudiculata	$n(2x) = 40$	–	–	–	15	2	–
subspicata	$n(3x) = 60$	–	–	1	2	10	1

been determined from pollen mother cells. For instance:

species	PMC's	No. cells with x	2x	3x	4x	6x	8x	10x	12x
dooneri	n(x) = 20	3	24	-	23	-	-	-	-
deserti	n(x) = 20	2	10	-	34	2	-	-	-
cylindrica	n(x) = 20	-	1	-	4	19	2	2	1

For this reason, the mitotic studies were temporarily suspended until the true n numbers could be established for as many of the species as possible. It is planned to resume them as time permits.

The mode of origin of reduced, partially reduced, super-reduced, doubled, and partially doubled cells, apparently as a regular feature of roottip growth, is of considerable intrinsic cytological interest. Also, this phenomenon may be of some pertinence to breeding problems. In this regard, answers to three questions will be sought: (a) Do doubled or reduced cells ever give rise to spore mother cells and, hence, to gametes with aberrant numbers? The triploid trifasciata-deserti hybrids suggest that they do. (b) Is the apparent variation between species in extent and range of reduced and polyploid cells real or only due to insufficient sampling? (c) If real, are the differences correlated with physiological or other characters of either practical or taxonomic interest?

That the differences between species are both real and under genetic control is suggested by a comparison of trifasciata, deserti, and their diploid F_1 hybrid H51-10. Trifasciata had mostly diploid cells, while deserti actually had more 4x than 2x cells in roottips. Counts for H51-10 were as follows:

2x - 64 cells
x - 1 cell
< x - 5 cells

Hence, the hybrid had mostly diploid cells like trifasciata, but probably a higher frequency of reduced cells than either parent.

The question has frequently arisen, in discussion of these results, as to whether the variation in number may not have been induced by the PDB pretreatment. PDB is, of course, a C-mitotic agent and, hence, could induce chromosome doubling. This explanation is thought to be ruled out by the following considerations:

(1). Variation in number (including 1=x, 1=3x, and 3=4x cells) was first noted in early preparations of metallica roottips which had not been treated with PDB. From time to time, untreated "control" roots were fixed and studied from several other species. It is, of course, much more difficult to find unbroken cells sufficiently well spread for accurate counts in the untreated roots, but the difference between x, 2x, 4x, 6x, etc., nuclei is great enough so that ploidy of most dividing cells can be estimated. Approximately the same range of numbers was found in these roottips as in PDB-treated roots of the same species.

(2). The length of exposure to PDB was in the range known, in other plants, to give a high proportion of C-metaphases without appreciable numbers of C-anaphases (i.e. doubled cells). That the length of exposure was correct for Sansevieria was evidenced by (a) the frequent finding of normal (unaffected) anaphases, and (b) by the almost total absence of (easily recognized) early C-anaphases. Only three of the latter were seen in over 200 slides, although a careful search was made for them in every slide from which counts were recorded.

(3). Even had an occasional 4n cell been due to doubling following PDB treatment, the length of exposure was certainly insufficient to have produced the range of numbers found, for instance, in cylindrica. Moreover, neither PDB nor any other substance known to me has been found to induce series of reduced and unevenly doubled numbers (e.g., n, 3n, 5n, 6n, etc., from 2n).

The conclusion seems warranted, therefore, that the variation in number is a property of the species, and not of the PDB pretreatment, and that the continued use of the PDB technique is justified to insure a large number of countable plates.

Table 1. Summary of meiotic data from 11 species of Sansevieria.

Species	Ploidy	Pairing at metaphase I
Group I		
cylindrica	2x	20 II
deserti	2x	20 II
suffruticosa	2x	20 II
canaliculata	4x	1-5 IV's, rest II's
Group II		
angustiflora	2x	20 II
liberica	ca. 4x	irregular, I's, III's and IV's present
subspicata	6x	complex, but regular, IV's and VI's present
Group III		
trifasciata	2x	20 II
metallica	2x	20 II
dooneri	2x	20 II
parva	2x	20 II

Table I. Summary of meiotic data from 11 species of *Amaryllis*.

Species	Ploidy	Pairing at metaphase I
Group I		
cylindrica	2x	20 II
desertii	2x	20 II
multiflora	2x	20 II
reginulata	4x	1-5 IV's, rest II's
Group II		
angustifolia	2x	20 II
bifurcata	ca. 4x	irregular, I's, III's and IV's present
subplicata	6x	complex, but regular, IV's and VI's present
Group III		
bifurcolata	2x	20 II
metallica	2x	20 II
cooperi	2x	20 II
parva	2x	20 II

Table 2. Summary of meiotic data from five types of species hybrids.

Hybrid	Parents	Ploidy	Pairing at metaphase I
H51-10	trifasciata X deserti	2x	20 II
H54-3	trifasciata X deserti	3x±	irregular, many III's
H54-7	" "	3x±	" " "
H54-8	" "	3x±	" " "
H54-9	" "	3x±	" " "
H51-1	trifasciata X liberica	3x-	irregular, III's & I's
H51-2	" "	3x+	more than 20 associations, III's & I's
H51-8	trifasciata X parva	2x	20 II most cells
H52-173	parva X deserti	2x	20 II (2 cells with I's).

Table 2. Summary of meiotic data from five types of crosses of cycads in Florida.

Hybrid	Parents	Ploidy	Pairing at metaphase I
HCl-10	Lepidozamia X Macrozamia	2x	20 II
HCl-3	Lepidozamia X Macrozamia	3x+	Irregular, many III's
HCl-7	" "	3x-	" " "
HCl-8	" "	3x-	" " "
HCl-9	" "	3x-	" " "
HCl-1	Lepidozamia X Bowenia	3x-	Irregular, III's & I's
HCl-2	" "	3x+	more than 20 associations, III's & I's
HCl-3	Lepidozamia X Zamia	2x	20 II most cells
HCl-173	Zamia X Macrozamia	2x	20 II (2 cells with I's).